SEMI-PREPARATIVE SUPERCRITICAL FLUID EXTRACTION/FRACTIONATION

R.M. Campbell and M.L. Lee

Department of Chemistry, Brigham Young University, Provo, Utah 84602 USA

Supercritical fluids have been used with considerable success both as mobile phases in chromatography and as solvents in extraction processes. While packed columns have been used in chromatography with greatly reduced analysis times (1), ease of solvent removal has been one of the major benefits of extractions with supercritical CO_2 (2). The supercritical fluid extraction of caffeine from coffee and of nicotine from tobacco are only a few of the many uses of supercritical fluids for separations which have been reported. These have been reviewed previously (2). In several studies, packed columns were used to improve the separation of closely related compounds. For example, supercritical fluid fractionation (SFF) methods were used to isolate several polycyclic aromatic hydrocarbons (PAH) in an automobile exhaust extract (3). These studies have shown that the ability to instantaneously vary the solvating power of a supercritical fluid by changing its pressure (or density) can be used to great advantage. Using the selectivity of the mobile phase, compounds were resolved in SFC which would have required many more theoretical plates to resolve in gas chromatographic systems (4). This mobile phase selectivity can only be preserved by avoiding large pressure drops across the column which can occur in columns packed with very small particles or in systems operated at very high flow rates (5). In the present study, columns packed with silica materials of intermediate particle sizes (30 to 70 μm) were used to prevent large pressure drops and allow dynamic pressure programming to achieve semi-preparative scale separations.

EXPERIMENTAL

A supercritical fluid extraction/fractionation system was constructed to provide separations and fraction collection on a semi-preparative scale. A schematic diagram of this system is shown in Figure 1. The system included a 375-mL syringe pump (Isco, Lincoln, NE) modified for pressure control at flow rates of up to 8 mL/min (liquid), a chromatographic oven (Varian, Walnut Creek, CA), and four 125-mL fraction collection vessels which were fitted with cooling jackets. During fraction collection, the vessels were cooled to 3 ± 2°C *via* a circulating cooling bath (Grant Science/Electronics, Dayton, OH). A six-port switching valve (Valco Instrument Co., Houston, TX) was used to collect successive fractions in different collection vessels. The collection vessels were pressurized with N_2 from a high pressure tank in conjunction with appropriate valving. A micrometering valve (Autoclave Engineering, Erie, PA) was used to control the flow when the effluent was vented directly to atmosphere. An extraction column (10 cm x 4.6 mm i.d.) and a separation column (25 cm x 4.6 mm i.d.) were placed in the oven, and effluents were monitored with a UV-absorbance detector (Hitachi, Model 100-10, Tokyo, Japan) equipped with a high pressure cell (Hewlett-Packard, Avondale, PA). All parts of the extraction/fractionation apparatus were constructed of stainless steel.

A coal tar was fractionated by adding 1 mg of the tar in 50 μL of methylene chloride to the top of the extraction column which had been dry-

packed with 40-63 µm silica (Sigma, No. S-0507, St. Louis, MO). The column end fittings (equipped with 2 µm frits) were tightened and the column was installed in the oven by tightening the appropriate fittings. The separation column was dry-packed with NH_2-Adsorbosil (Applied Science, Deerfield, IL, 30 - 70 µm). The oven temperature was raised to 40°C and held there for the duration of the fractionation. The pressure was brought to 72 atm, then immediately raised to 95 atm at 8 atm/min. The pressure was held at 95 atm until phenanthrene began to elute (Fraction 3), whereupon it was raised at 1.5 atm/min to 98 atm. As soon as the fluoranthene/pyrene peak (Fraction 4) started eluting, the pressure was again programmed at 1.5 atm/min to 130 atm. At this point, as the chrysene peak (Fraction 5) was finishing, the pressure was programmed at 5 atm/min to 198 atm and held for about 10 min. Fractions were collected as marked on the chromatogram in Figure 3. Fractions were analyzed by capillary gas chromatography with an HP 5880 gas chromatograph equipped with a 20 m x 0.2 mm i.d. fused silica capillary column coated with SE-54 stationary phase which was crosslinked with azo-t-butane.

RESULTS AND DISCUSSION

In 1977, Wise et al. (6) reported the separation of PAH according to number of aromatic rings using an HPLC system with a chemically bonded aminosilane stationary phase. This was very important because each of the ring-number cuts could then be analyzed by reversed phase HPLC, which provides resolution of closely related isomers. More recently, ring-number fractions of complex mixtures of PAH have been sought for analysis by GC with a liquid crystalline stationary phase, which exhibits excellent selectivity for the separation of geometric isomers (7,8).

Figure 2 shows the UV chromatogram of the fractionation of a number of standard PAH using the SFF system. A similar pressure program was used to fractionate a coal tar sample as shown in Figure 3. Figure 4 shows the capillary gas chromatograms of typical fractions of the coal tar extract obtained on the SFF system. The polar amino bonded phase provided good selectivity for separations by ring number. Alkylated species tended to elute at or near the same time as their parent compounds, while compounds of different ring structure were widely separated.

Samples were best introduced into the SFE system by applying the solutes in a small amount of solvent to the head of the column with a syringe. This method resulted in narrower bands than are obtained when the sample is distributed over the entire extraction column. An external sample loop injection valve was not used in the system because at the lower pressures used at the beginning of a fractionation run, all sample components were not dissolved, resulting in plugging of the frits at the head of the separation column.

Adsorbents of intermediate particle size (30-70 µm) were found to give the best separations. The trade-off between column efficiency and pressure drop was at or near optimum with this particle size. Smaller particles resulted in large pressure drops, while larger particles resulted in poor efficiency and large column dead volumes.

In addition, standard compounds were used to study the effect of mobile phase density on resolution of closely related compounds. The resolution of biphenyl and acenaphthalene was measured at a number of different mobile phase densities. Results of this study are listed in Table I. It was found that, in general, the resolution of a pair of closely related isomers could be improved by as much as 100% by decreasing the mobile phase pressure at

constant temperature on a given column. However, it was also concluded that the particle size of the packing in the column has a large effect on resolution as well. Column efficiency, which is solely dependent on the particle size of the column packing material, may have a greater effect on resolution than the selectivity of the mobile phase. Also, by going to smaller particle diameter of the column packing, analysis times can be shortened. However,

Table 1. Resolution of Acenaphthalene and Biphenyl at Various Pressures of CO_2 at 40°C and using a 35-70 μm NH_2-silica column.

Pressure	Resolution
85	1.1
90	1.1
100	0.8
120	0.9
140	0.9
160	0.9
180	0.7
198	0.5

large pressure drops across columns packed with very small particles cause significant selectivity losses. For some solutes, the gain in efficiency is greater than the loss in selectivity on going to a smaller diameter packing. It was concluded that particles in the 35-80 μm range were best for the separations we have attempted thus far.

UV monitoring of the column effluents was essential to enable precise cuts during fractionation on a routine basis as well as during development. Solutes in the fractions were best collected by cooling the collection vessels to 2 to 5°C. This created a two-phase (gas/liquid) region in the collection vessels, causing the solutes to precipitate out of the gas phase. In this way, the solutes were removed from the CO_2 before it decompressed through the fused silica restrictors. The cooling was not enough to cause precipitation of very volatile solutes such as naphthalene. Therefore, fractions containing very volatile components were collected by bubbling the effluents through methylene chloride or n-pentane.

With our current pumping system, the maximum sample capacity appears to be about 10 to 20 mg per run. Figure 5 shows the UV SFF chromatogram of 8 mg of coal tar extract obtained on a 25 cm x 6.2 mm i.d. column with a 10 cm x 6.2 mm i.d sample introduction column. The NH_2-Adsorbosil stationary phase was used in this case as well. Fractions were again collected as marked on the chromatogram. Typical analysis times for separations of components ranging from 2 to 5 rings was between 1 and 1.5 h.

Overall, it was found during this study that semi-preparative supercritical fluid fractionation shows great potential for high quality separations with easy solvent removal.

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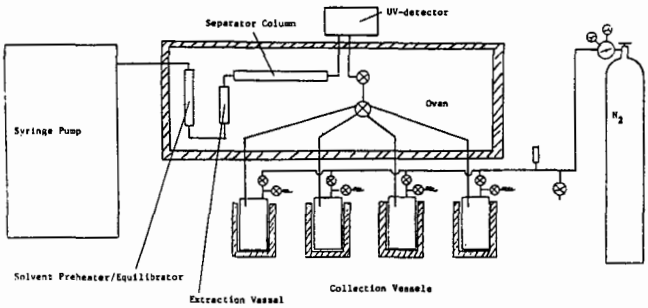

Figure 1. Schematic diagram of the supercritical fluid extraction/fractionation system.

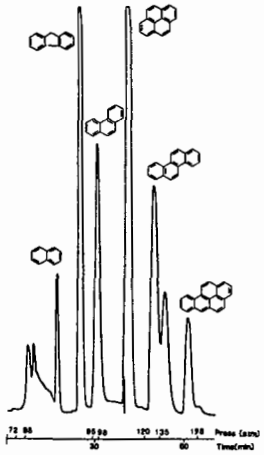

Figure 2. UV chromatogram of the supercritical fluid fractionation of a number of standard compounds with CO_2 at 40°C.

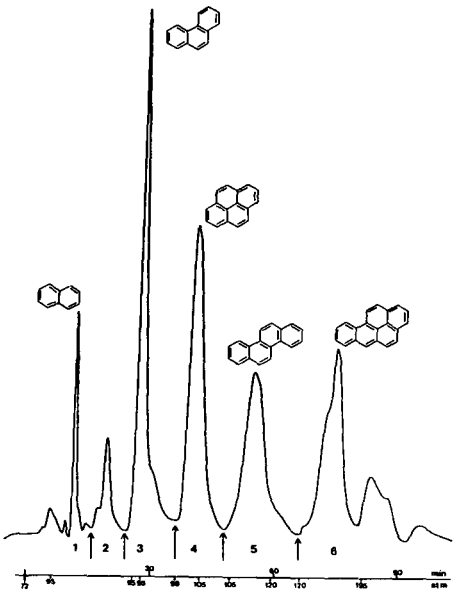

Figure 3. UV chromatogram of the supercritical fluid fractionation of 1 mg of a coal tar extract with CO_2 at 40°C. The numbers 1-6 indicate the fractions that were collected.

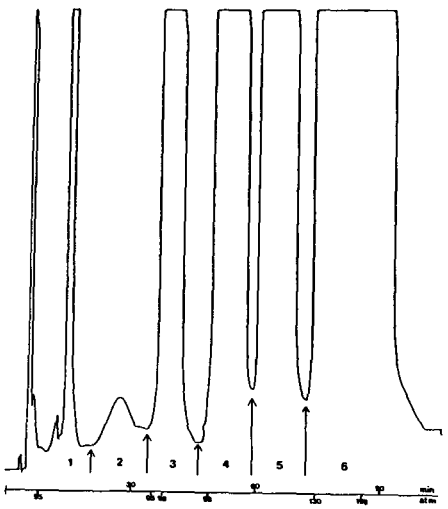

Figure 5. UV chromatogram of the supercritical fluid fractionation of 8 mg of the coal tar extract with CO_2 at 40°C.

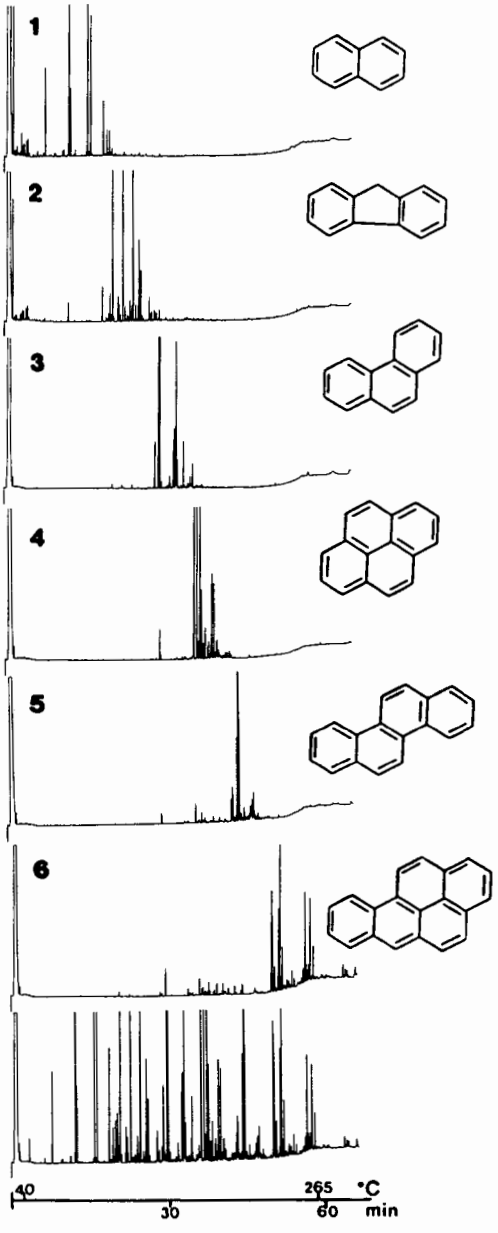

Figure 4. Capillary gas chromatograms of the six fractions collected during the supercritical fluid fractionation of the coal tar extract. The numbers refer to the fractions shown in Figures 3 and 5. The bottom chromatogram shows the total unfractionated coal tar for comparison.